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Human T Helper Cells Specific for Antigens of Typhus Group Rickettsiae Enhance Natural Killer Cell Activity In Vitro

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The peripheral blood mononuclear cells (PBMC) from 5 individuals immune to typhus group rickettsiae and from 13 nonimmune individuals were stimulated in vitro for 7 days with typhus group rickettsial antigen (TGRA). At the end of day 7, lysis of the natural killer (NK)-susceptible target K562 by these PBMC was determined. As controls, PBMC from both groups of donors were cultured in vitro for 7 days without antigen or were freshly isolated, and lysis of the K562 target was determined. There was no significant difference between the level of NK activity in freshly isolated PBMC from immune and nonimmune donors. PBMC from immune donors which were stimulated with antigen for 7 days exhibited significantly greater NK activity than did the control population, which was cultured for 7 days without antigen. PBMC from immune donors which were stimulated with TGRA demonstrated significantly higher NK activity than the same PBMC stimulated with antigen derived from an antigenically unrelated rickettsia, *Coxiella burnetii*. There was no significant difference, however, in the level of NK activity of nonimmune antigen-stimulated PBMC compared with that of the same PBMC population cultured without antigen. Most of the antigen-stimulated NK activity was mediated by Leu-11-positive cells as determined by electronic cell sorting. The ability of TGRA to sustain the NK activity of PBMC from immune donors was abolished when the T4/Leu-3-positive population of lymphocytes was eliminated by positive or negative selection prior to antigen stimulation. The ability of TGRA to sustain the NK activity of PBMC from immune donors was also significantly decreased in the presence of antibodies against human interleukin-2. The results suggest that the activity of human NK cells can be sustained in vitro by antigen-specific T helper cells and that the effect of the T helper cell is mediated, at least in part, by interleukin-2. Reprints. (AW)

Rickettsia typhi and *Rickettsia prowazekii*, the etiologic agents of endemic and epidemic typhus, respectively, are obligate intracellular bacteria transmitted by arthropod vectors (23). Studies in mouse and guinea pig models have shown that both humoral and cellular mechanisms are important in the immunologic defense against these agents (6, 13). In humans, a variety of immunologic responses to these organisms have also been described. We have recently shown that in vitro stimulation of peripheral blood mononuclear cells (PBMC) from typhus group rickettsia-immune donors with typhus group rickettsiae-derived antigen (TGRA) results in the generation of lymphokine-activated killer cells which can lyse targets infected with typhus group rickettsiae (5). It has also been demonstrated that in vitro stimulation of PBMC with TGRA results in the production of specific anti-typhus group rickettsia antibody and that this antibody production can be regulated by populations of helper and suppressor lymphocytes (12). Since the stimulation of PBMC with either alloantigens or viral antigens results in the stimulation of a variety of nonspecific effectors, such as natural killer (NK) cells and lymphokine-activated killer cells, as well as antigen-specific cytotoxic effectors (11, 17), we examined the ability of rickettsia antigen to affect NK cell activity *in vitro*.

In the present study, we show that PBMC from typhus group rickettsia-immune donors demonstrated significant NK activity when stimulated in vitro with TGRA compared with NK activity from nonimmune-donor PBMC which were stimulated in the identical fashion. We further show that the cell responsible for most of this NK activity is Leu-11-positive and that stimulation of this Leu-11-positive NK cell is carried out by a T4/Leu-3-positive helper. We also show that the T-helper-cell stimulation of NK cells is dependent on human interleukin 2 (IL-2). We believe these results are important since they demonstrate for the first time that the activity of human NK cells can be sustained in vitro by an antigen-specific T helper.

MATERIALS AND METHODS

Lymphocyte donors. PBMC were obtained from 18 individuals in the present study. Five donors had serologic evidence of infection with either *R. typhi* or *R. prowazekii*, as determined by enzyme-linked immunosorbent assay (7), with or without a history of clinical rickettsial disease. The other 13 individuals had neither serologic nor historical evidence of prior illness with typhus group rickettsiae.

Preparation of antigens. Suspensions of Renografin-76 (E. R. Squibb & Sons, Princeton, N.J.) density gradient-purified *R. typhi* Wilmington grown in chicken yolk sacs (25) were passed through a French pressure cell at 20,000 lb/in^2 twice, and the crude extract was centrifuged at 8,000 rpm for 5 min in a Sorvall SS-34 fixed-angle rotor to remove intact cells. Formalin was then added to a final concentration of 0.2%, and after 48 h at room temperature the antigen suspension was dialyzed for 72 h at 4°C against 500 to 1,000 volumes of RPMI 1640 (GIBCO Laboratories, Grand Island, N.Y.) containing a 1% penicillin-streptomycin solution (GIBCO). After dialysis, the antigen was diluted to the appropriate working concentrations in RPMI 1640 and stored at −60°C. Control whole-cell antigen derived from *Coxiella burnetii* was kindly provided by Jim C. Williams, U.S. Army Medical Research Institute for Infectious Diseases, Frederick, Md.

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TABLE 1. Mean percent specific lysis of the K562 target from typhus group rickettsiae-immune and nonimmune donors at all effector/target ratios

PBMC	% Lysis compared in effector/target ratios (mean ± SD)		
	25:1	6:1	1:1
Immune-donor cells			
Cultured in vitro with antigen	48.8 ± 11.8	28.8 ± 12.9	9.6 ± 4.7
Cultured in vitro without antigen	2.1 ± 1.8	0.9 ± 1.1	1.1 ± 1.4
Freshly isolated	35.0 ± 15.2	18.4 ± 7.1	5.3 ± 2.5
Nonimmune-donor cells			
Cultured in vitro with antigen	11.3 ± 10.6	4.3 ± 3.8	1.6 ± 1.2
Cultured in vitro without antigen	3.2 ± 1.2	2.4 ± 1.2	2.2 ± 1.4
Freshly isolated	49.6 ± 10.0	25.8 ± 8.0	8.9 ± 3.2

The method for preparing *C. burnetti* has been reported previously (24).

In vitro stimulation of PBMC. PBMC were obtained by Ficoll-Hypaque centrifugation of diluted peripheral venous blood as previously described (3). PBMC were suspended at a concentration of 10^6 cells per ml in RPMI 1640 supplemented with 10% heat-inactivated pooled human AB serum (Flow Laboratories, Inc., McLean, Va.), 1% glutamine (GIBCO), 1% penicillin-streptomycin, and 2-mercaptoethanol at a concentration of 5×10^{-5} M. A total of 10^7 cells were incubated with 80 μg of TGRA protein in 8 ml of medium in tissue culture flasks (type 25100; Corning Glass Works, Corning, N.Y.) in a humidified atmosphere containing 5% CO_2 in air for 7 days. As a control in two donors, 10^7 PBMC were stimulated with 40 μg of protein of *C. burnetii* antigen in 8 ml of medium and incubated in a humidified atmosphere containing 5% CO_2 in air for 7 days. This concentration of *C. burnetii* antigen has been shown to produce maximum proliferation of PBMC derived from *C. burnetii*-immune individuals (J. C. Williams, personal communication).

Assay for NK cell activity. The target cell used in all assays was the NK-susceptible K562 myeloid cell line (16) obtained from the American Type Culture Collection. Cytotoxicity assays were performed in 96-well V-bottom microtiter plates (PGC Scientific, Gaithersburg, Md.), and each effector/target ratio was determined in triplicate. Target cells were harvested, suspended in 0.2 ml of medium, and radiolabeled with 240 μCi of $Na_2{}^{51}CrO_4$ for 60 to 90 min at 37°C. The target cells were washed three times, viable cells were counted by trypan blue exclusion, and 10^4 viable target cells (in 50 μl of medium) were added to various numbers of effector cells (in 100 μl of medium). After the microtiter plates were incubated for 4 to 6 h at 37°C, 50 μl of supernatant was removed from each well. In addition, each assay contained target cells incubated with medium without added effector cells (spontaneous release) and target cells incubated in 5% Triton X-100 (maximum release). Percent specific cytotoxicity was calculated as follows: % specific lysis = [(experimental release − spontaneous release)/(maximum release − spontaneous release)] × 100. Standard error of the mean is given in all cases. The ratio of spontaneous release to maximum release was always less than 0.15. The data was analyzed by using the Student *t* test.

Cell sorting. TGRA-stimulated PBMC from two typhus group rickettsia-immune donors were selected for the presence or absence of surface Leu-11 by electronic cell sorting with a fluorescence-activated cell sorter (FACS II; Becton Dickinson, Mountain View, Calif.) (1). Similarly, PBMC from another typhus group rickettsia-immune donor were selected, prior to stimulation with TGRA, for the presence or absence of Leu-3 by electronic cell sorting. Ten microliters of anti-Leu-11a monoclonal antibody or anti-Leu-3a monoclonal antibody (Becton Dickinson Monoclonal Center, Inc., Mountain View, Calif.) were added to each pellet of 10^7 effector cells. Cells were then incubated for 1 h at 4°C and washed twice with Hanks balanced salt solution without phenol red and containing 1% fetal bovine serum. Cells were passed through the FACS at a rate of 2,400 total light scatter signals per s. Cells were selected for positive and negative fluorescence and for viability by light scatter analysis. The cells were collected over a 6-h period in 5-ml tubes at 4°C containing 0.5 ml of fetal bovine serum. A portion of cells was not sorted and served as the unseparated control. After completion of the sorting, 10^4 cells of each fraction were reanalyzed in the FACS to assess purity.

Depletion of the T4-positive cell population with monoclonal antibody and complement. Before in vitro stimulation with TGRA described above, PBMC were suspended in RPMI 1640 at a concentration of 2×10^7/ml, and 0.2 μg of OKT4 monoclonal antibody (Ortho Diagnostics, Inc., Raritan, N.J.) was added to 10^7 cells in a total volume of 0.5 ml. After incubation at 4°C for 1 h, the cells were washed twice and suspended to a concentration of 10^7/ml in baby rabbit complement (Pel-Freez Biologicals, Rogers, Ariz.) which had been diluted 1:2 in RPMI 1640. After being washed twice, the cells were treated with a second course of monoclonal antibody followed by addition of complement and an additional wash.

The effectiveness of negative selection with OKT4 monoclonal antibody and complement was analyzed by direct immunofluorescence measured by flow microfluorometry with the FACS II as previously described (1). OKT4 antibody conjugated to fluorescein isothiocyanate (Ortho Diagnostics) was used for cell staining.

Effect of antibody against human IL-2 on antigen-maintained NK cell activity. Affinity-purified rabbit immunoglobulin G anti-human IL-2 was obtained from Collaborative Research, Inc., Lexington, Mass. (no. 40012), and normal rabbit serum was obtained from Pel-Freez Biologicals (no. 31199-0). Serial 10-fold dilutions from 1:10 to 1:1,000 were made in triplicate in round-bottom microtiter plates (no. 76-013-05); Flow Laboratories) containing 100 μl of RPMI 1640 supplemented with 10% pooled human AB serum. A total of 2×10^5 PBMC in 100 μl each from two typhus group rickettsia-immune donors (in two separate experiments) were then added to each well, in addition to TGRA at a final concentration of 10 μg/ml. As a control, PBMC were cultured with antigen but without any rabbit immunoglobulin. All PBMC were cultured for 7 days before assaying for NK activity.

RESULTS

Assessment of NK activity of PBMC from immune or nonimmune donors under different conditions. PBMC from 5 immune and 13 nonimmune donors were (i) incubated in vitro for 7 days in the presence of TGRA, (ii) incubated in vitro for 7 days in the absence of TGRA, or (iii) freshly isolated with adherent cell populations removed by two 1-h incubations in plastic tissue culture flasks (to mimic deple-

tion steps of 7-day-cultured cells). The PBMC were then tested for their ability to lyse the NK-susceptible target K562. Table 1 shows the mean percent specific lysis for both immune and nonimmune donors at effector/target ratios of 25:1, 6:1, and 1:1. There was no significant difference between the lysis of the K562 targets by freshly isolated PBMC from immune donors and the lysis by freshly isolated PBMC from nonimmune donors at all effector/target ratios ($0.21 \geq P \geq 0.14$). Although PBMC from immune donors showed significantly greater NK activity when placed in culture and stimulated with antigen than when placed in culture without antigen at all effector/target ratios ($P \leq 0.05$), PBMC from nonimmune donors showed no greater NK activity when placed in culture and stimulated with antigen compared with the same cells placed in culture without antigen at all effector/target ratios ($0.60 \geq P \geq 0.09$). The level of NK activity exhibited by the PBMC of immune donors when stimulated with antigen was not significantly different than the base-line NK activity of the freshly isolated immune PBMC at all effector/target ratios ($0.37 \geq P \geq 0.28$). Antigen-stimulated PBMC from nonimmune donors, on the other hand, showed significantly less NK activity than did the PBMC freshly isolated from the same nonimmune donors at all effector/target ratios ($P \leq 0.001$). NK activity was also significantly higher in antigen-stimulated PBMC from immune donors than in antigen-stimulated cells from nonimmune donors at all effector/target ratios ($P \leq 0.005$).

PBMC from two typhus group rickettsia-immune donors were also stimulated in vitro for 7 days with *C. burnetii*-derived antigen, as well as with TGRA. The ability of these antigen-stimulated PBMC to lyse the K562 target was then compared with the lysis of the K562 target by autologous, freshly isolated PBMC and by autologous PBMC cultured in vitro for 1 week, without antigen (Table 2). In both donors, PBMC stimulated with TGRA had significantly higher NK activity at all effector/target ratios than did the *C. burnetii* antigen-stimulated PBMC ($P < 0.001$). Results with freshly isolated PBMC and unstimulated cultured PBMC were similar to those in Table 1. At the higher effector/target ratios of 25:1 and 12:1, PBMC from one donor which were stimulated with *C. burnetii* antigen had significantly higher NK activity than did PBMC cultured without antigenic stimulation ($P < 0.001$). It has been shown previously, however, that the *C. burnetii*-derived antigen can have a mitogenic effect on the PBMC from some nonimmune individuals (J. C. Williams, personal communication).

TABLE 2. NK activity of PBMC from typhus group rickettsia-immune donors stimulated with TGRA or *C. burnetii*-derived antigen

PBMC	% NK activity compared in effector/target ratios (mean ± SD)		
	25:1	12:1	6:1
Expt 1			
Cultured in vitro with TGRA	48.5 ± 1.4	31.6 ± 6.0	11.5 ± 1.3
Cultured in vitro with *C. burnetii* antigen	0 ± 0	0 ± 0	0 ± 0
Cultured in vitro without antigen	0 ± 0	0 ± 0	0 ± 0
Freshly isolated	15.4 ± 2.3	4.3 ± 1.6	0 ± 0
Expt 2			
Cultured in vitro with TGRA	78.9 ± 2.5	61.8 ± 2.4	36.9 ± 2.0
Cultured in vitro with *C. burnetii* antigen	20.6 ± 0.8	9.6 ± 0.8	4.0 ± 0.6
Cultured in vitro without antigen	0 ± 0	0 ± 0	0 ± 0
Freshly isolated	58.4 ± 2.6	34.9 ± 2.5	25.8 ± 0.8

TABLE 3. Phenotypic characterization by electronic cell sorting of antigen-stimulated PBMC with NK activity

PBMC	Lysis of K562 (mean ± SD)[a]	Change in lysis (P)[b]
Expt 1		
Unsorted	27.1 ± 2.0	
Unsorted + anti-Leu-11a	25.9 ± 3.5	−4.4 (0.69)
Leu-11 positive	42.2 ± 2.9	+62.9 (0.03)
Leu-11 negative	15.0 ± 3.5	−42.0 (0.01)
Expt 2		
Unsorted	43.4 ± 1.3	
Unsorted + anti-Leu-11a	41.8 ± 0.4	−3.6 (0.29)
Leu-11 positive	43.6 ± 2.2	+4.1 (0.42)
Leu-11 negative	13.4 ± 0.8	−67.9 (0.001)

[a] Effector/target ratio of 3:1.

[b] Decreases and increases are indicated by minus and plus signs, respectively.

Phenotypic characterization of cells mediating the lysis of the NK-susceptible target. Since lymphocytes other than NK cells can lyse NK-susceptible targets (14), it was unclear whether the observed enhancement of NK activity in the PBMC from immune donors stimulated with rickettsial antigen was mediated by NK cells or by a different population of lymphocytes. To answer this question, we stimulated PBMC from two immune donors with rickettsial antigen, and then selected for the presence or absence of surface Leu-11 by electronic cell sorting with the FACS II. Two unsorted cell populations were used as controls, one with anti-Leu-11a antibody added, and one without antibody. The Leu-11-positive fraction demonstrated no decrease in NK activity compared with that of the unsorted populations, whereas the Leu-11-negative population demonstrated a significant decrease in NK activity compared with that of the unsorted populations (Table 3). Analysis of the two populations by flow microfluorometry showed that each was approximately 95% pure. In addition, as reported previously (9), binding of anti-Leu-11a antibody to NK cells had no significant effect on their ability to lyse the K562 target.

Sustained NK activity mediated by TGRA is dependent on a T4/Leu-3-positive helper cell. Because significantly higher NK activity could be found when the in vitro cultures of PBMC from immune individuals were stimulated with TGRA but not with *C. burnetii* antigen, it was evident that there was an antigen-specific component involved in this phenomenon. Since NK cells can be activated by various nonspecific factors (18), it seemed unlikely that there would be any inherent differences in the NK cells of immune donors which would enable these cells to respond specifically to an antigen. Instead, we hypothesized that maintenance of the NK cell activity was being mediated by an antigen-specific helper cell. To test this hypothesis, we eliminated the T4-positive population of lymphocytes from the PBMC of a typhus group rickettsia-immune donor with OKT4 antibody and complement prior to stimulating the PBMC with rickettsial antigen. As controls, we used antigen-stimulated PBMC which were treated with complement only, and antigen-stimulated PBMC which were treated with neither antibody nor complement. Elimination of the T4-positive population significantly decreased the observed

TABLE 4. Effect of depletion of T4-positive cells upon NK cell activity induced by TGRA

Treatment	Effector/target ratio	% Lysis of K562 (mean ± SD)	% Change in lysis (P)[a]
None	12:1	57.6 ± 0.5	
	3:1	38.7 ± 5.2	
OKT4 + C1	12:1	7.3 ± 0.9	−87.3 (<0.001)
	3:1	2.9 ± 0.5	−92.5 (0.02)
C1 alone	12:1	58.4 ± 1.5	+1.3 (0.62)
	3:1	40.7 ± 2.8	+5.2 (0.69)

[a] Decreases and increases are indicated by minus and plus signs, respectively.

lysis of the K562 target by 87.3 and 92.5% at effector/target ratios of 12:1 and 3:1, respectively (Table 4). Treatment of the effector population with complement alone, however, resulted in no significant change in the lysis of the K562 target.

We obtained similar results when we eliminated the helper cell population by electronic cell sorting. After selecting for the presence or absence of Leu-3a from the PBMC of a typhus group rickettsia-immune donor, we stimulated Leu-3a-negative cells and unsorted populations in vitro for 7 days with TGRA. The ability of the Leu-3a-negative population to lyse the K562 target was significantly decreased (Table 5). At effector/target ratios of 12:1 and 3:1, respectively, lysis was decreased by 74.1 and 100% compared with that of the stained and unsorted population, and lysis was decreased by 71.6 and 100% compared with that of the unstained and unsorted population. The Leu-3a-negative population was approximately 95% pure when analyzed by flow microfluorometry.

Sustained NK-activity induced by TGRA is mediated by IL-2. PBMC from two typhus group rickettsia-immune donors were cultured in vitro for 7 days in medium containing TGRA and serial 10-fold dilutions of rabbit immunoglobulin G against human IL-2 or normal rabbit serum. As a control, PBMC were incubated with antigen alone. The ability of TGRA to sustain NK activity in both donors was significantly decreased by rabbit anti-human IL-2 at dilutions of 1:10 and 1:100 ($P \leq 0.04$) compared with NK activity in PBMC stimulated with antigen in the absence of such antibody (Table 6). At a 1:1,000 dilution of the rabbit anti-human IL-2, there was no significant decrease ($P \geq 0.08$) in the ability of rickettsia antigen to sustain NK activity. In addition, incubation of PBMC stimulated with TGRA in the presence of normal rabbit serum resulted in no decrease in the observed NK activity compared with that of PBMC stimulated with antigen alone ($P \geq 0.30$). There were no significant differences in effector viability between any of the groups, and the final effector/target ratio was approximately 40:1.

DISCUSSION

NK cells have been characterized as a population of large granular lymphocytes (19) which are capable of lysing a limited range of tumor cell targets (8). Although NK cells have been shown to possess a variety of surface antigens which they share with other functional classes of lymphocytes (9), the Leu-11 antigen, which represents a portion of the Fc receptor for aggregated immunoglobulin G, is found on NK cells and neutrophils only (15). It has also been demonstrated that almost all spontaneous NK activity resides within the Leu-11-positive population of cells (9).

When human PBMC are cultured in vitro in autologous plasma, the level of NK activity generally decreases to insignifcant levels within a period of 1 week (10). In the present study, we demonstrated that the NK activity of PBMC from a group of individuals with known immunity to an infectious agent was maintained during culture in vitro with antigenic material derived from that infectious agent. We also demonstrated that most of the NK activity following antigen stimulation took place in the Leu-11-positive population. Since this significantly high level of NK activity was not seen when PBMC from nonimmune individuals were similarly stimulated or when PBMC from immune individuals were stimulated with an unrelated rickettsial antigen against which they had no immunity, we hypothesized that the Leu-11-positive population was being stimulated by a helper cell that was responding specifically to the typhus antigens. Indeed, by eliminating the T4-positive population of cells before stimulating PBMC with antigen, no significant NK cell activity was observed. Although it has been demonstrated that the morphological appearance of some T4-positive cells is similar to that of NK cells, and that such cells can bind to appropriate NK-susceptible targets, the T4-positive cells are nevertheless incapable of lysing these targets (21). It is most certain, therefore, that the elimination of the T4-positive population resulted in the loss of a regulatory cell rather than a functional effector. Britten et al. (4) have reported similar findings in a rat model. They were able to demonstrate that NK cell activity in the rat can be regulated by a population of antigen-specific helper cells primed with *Mycobacterium bovis* BCG.

Both IL-2 and gamma interferon (18) are known to enhance the NK activity of PBMC cultured in vitro. Although there is some controversy over whether the enhanced NK activity caused by stimulation with IL-2 is mediated by the release of gamma interferon or is a direct effect of the IL-2 alone (20, 22), it was recently demonstrated that the NK activity of both Leu-11-positive and Leu-11-negative fractions of PBMC can be enhanced by pretreating the cells for 18 h with IL-2 (18). Further, it was demonstrated that IL-2 augments NK activity after in vitro stimulation for 18 h in the absence of any significant proliferation (20). In the present study, incubation of antigen-stimulated PBMC with appropriate concentrations of rabbit immunoglobulin G against

TABLE 5. Effect of depletion of Leu-3a-positive cells by cell sorting on NK cell activity enhanced by TGRA

Effector group	Effector/target ratio	% Lysis of K562 (mean ± SD)	% Change in lysis (P)[a]
1. Unstained and unsorted	12:1	63.8 ± 4.4	
	3:1	27.2 ± 1.8	
2. Stained and unsorted	12:1	69.9 ± 3.3	
	3:1	33.3 ± 1.6	
3. Leu-3a negative	12:1	18.1 ± 0.3	−71.6 (0.001) compared with group 1; −74.1 (<0.001) compared with group 2
	3:1	0 ± 0	−100 (<0.001) compared with groups 1 and 2

[a] Decreases are indicated by minus signs.

TABLE 6. Effect of antibody against IL-2 on TGRA-stimulated NK activity

PBMC	% Lysis of K562 (mean ± SD)[a]			
		Antibody dilutions (% inhibition)		
		1:10	1:100	1:1000
Expt 1				
No antibody	77.7 ± 7.1			
Anti-IL-2		38.8 ± 4.3 (50.1)	48.2 ± 0.5 (37.8)	64.1 ± 1.0 (17.5)
Normal rabbit serum		76.2 ± 2.1 (1.9)	72.0 ± 3.2 (7.3)	69.8 ± 1.0 (10.1)
Expt 2				
No antibody	68.6 ± 12.3			
Anti-IL-2		26.5 ± 2.3 (61.3)	32.2 ± 2.4 (53.1)	54.2 ± 0.7 (21.0)
Normal rabbit serum		60.4 ± 4.5 (9.6)	66.1 ± 3.0 (3.6)	61.3 ± 3.0 (10.6)

[a] Effector/target ratio of approximately 40:1.

human IL-2 significantly decreasd the observed NK activity compared with that of antigen-stimulated PBMC in the absence of such antibody. The antibody against IL-2 may prevent direct stimulation of NK cells by IL-2 or may prevent activation and proliferation of the T helper cells necessary to stimulate the NK cells. The residual NK activity found in PBMC stimulated with antigen in the presence of antibody against human IL-2 may represent insufficient concentration of antibody or the effect of other soluble mediators such as gamma interferon. The removal of antigen-specific helper cells prior to stimulation with specific antigen probably results in the inability of the system to generate the quantities of IL-2 or other soluble mediators necessary to enhance NK activity. In the present study, the remaining NK activity in the Leu-11-negative fraction may represent the activity of contaminating Leu-11-positive cells, as well as lysis mediated by another cell population, such as lymphokine-activated killer cells.

Although we have previously demonstrated that typhus group rickettsia-infected targets can be lysed by lymphokine-activated killer cells (5), these same targets cannot be lysed by Leu-11-positive cells (unpublished observations). This is in contrast to targets infected with viral agents (2) which can be lysed by NK cells. The role of NK cells in immunity to typhus group rickettsiae is therefore unclear. Nevertheless, the maintenance of high NK activity by stimulation with TGRA provides a unique model for studying the interaction of NK cells with regulatory lymphocytes.

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